Official Title: Determination of Metabolomic Profile Differences in Sarcopenia Accompanying Fibromyalgia Syndrome

NCT Number: Not yet assigned (Initial Submission)

Document Date: Jan 25, 2023

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

This study was conducted in a single-center, cross-sectional observational design. The study complied with the Declaration of Helsinki and was approved by the Ankara Bilkent City Hospital No. 2 Clinical Research Ethics Committee with the number E2-22-1740. Informed consent was obtained from all individual participants included in the study. This study included 44 female patients aged 18-60 who were diagnosed with fibromyalgia according to the ACR (American College of Rheumatology) 2016 diagnostic criteria and applied to Ankara Bilkent City Hospital Physical Therapy and Rehabilitation Hospital outpatient clinics between May 2022 and December 2022 and 22 female healthy controls. Female volunteers who applied to Ankara Bilkent City Hospital Physical Therapy and Rehabilitation Hospital outpatient clinics for other reasons and who did not have any accompanying diseases such as fibromyalgia and sarcopenia constituted the healthy controls. Exclusion criteria include cognitive dysfunction; history of serious cardiac disease, COPD (Chronic Obstructive Pulmonary Disease), epilepsy, malignancy, neuromuscular disease, inflammatory rheumatic disease, endocrine disease and organ failure; currently pregnant; use of systemic glucocorticoid, ACE inhibitor, angiotensin receptor blocker, statin, sulfonylurea; male gender. Fibromyalgia is more common in women. Gender differences may affect metabolome results. That's why all participants in the study were women.

Demographic data, medication use and disease duration of the participants were recorded. Patients with fibromyalgia and healthy volunteers were screened for sarcopenia by using hand grip strength measurement with a hand dynamometer, muscle mass measurement

with bioimpedance analysis. According to EWGSOP2 (European Working Group on Sarcopenia in Older People) criteria, patients with hand grip strength below 16 kg were considered to have probable sarcopenia. 22 participants were included in the FMS PS- (Fibromyalgia Syndrome without Probable Sarcopenia) group and 22 participants were included in FMS PS+ (Fibromyalgia Syndrome with Probable Sarcopenia) group. The hand grip strength of all individuals in the healthy control group was over 16 kg.

Plasma samples were collected from all participants. Samples were centrifuged at 1500 rpm for 10 minutes and stored at -80 degrees to be used in metabolome analysis.

Measurement of plasma metabolomics

A mixture of methanol/water (90%:10% v/v) was added onto 100 µL of plasma. The samples were kept at -20°C overnight. The obtained samples were centrifuged at 15000 rpm for 10 minutes. Proteins precipitated and metabolites remained in the methanol/water mixture. The resulting metabolite mixture was placed in a vacuum centrifuge to remove methanol and water. 20µL methoxyamine (20mg/20µL) was added to the resulting metabolites. Afterwards, the samples obtained after derivatization with 80 µL MSTFA + 1% TMCS were analyzed by gas chromatography/mass spectroscopy (GC/MS).

Schimadzu qp2010 device was used in the analyses. In this study, FAME mix standards were used as standards in the system. DB-5MS stationary phase column (30 m+10m DuraGuard x 0.25mm i.d. and 0.25-µm) was used in the analysis. The oven temperature was fixed at 70°C for one minute, then increased to 320°C in increments of 10 degrees per minute and remained for 10 minutes. Afterwards, the system returned to its original temperature. The analysis took a total of 37 minutes. In the GC/MS system, the scanning range was 50-600 m/z.

The obtained GC/MS data were evaluated on the MS-DIAL metabolomics platform. Untargeted metabolomics studies have been conducted. Metabolites with a match score of 70

and above were accepted using the Kovats retention index database. In the pre-data analysis processes within the MS DIAL platform, peak identification, deconvolution and data alignment processes were primarily carried out. Subsequently, the structures of the metabolites were elucidated. Peak areas and heights were used in semi-quantitative analysis. Normalization processes were also carried out according to the total ion chromatogram. Two-tailed t-test was performed between experimental groups and significantly changed metabolites were identified. Metaboanalyst 4.0 platform and KEGG (Kyoto Encyclopedia of Genes and Genomes) database were used in pathway analyses.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) 22.0 Statistical Package Program and MS-DIAL metabolomics platform were used to evaluate the data. After determining whether the data showed normal distribution, statistical evaluation was made with appropriate parametric or non-parametric tests. The numerical data obtained are given as mean and standard deviation, and categorical data are given as number (n) and percentage (%). Descriptive statistical methods (Mean, Standard deviation, upper and lower values) were used when evaluating the data. After comparing the metabolome data between the three groups, cluster analysis was performed and pathway differences between the groups were revealed. The results were evaluated at the 95% confidence interval and the significance level was $p<0.05$.